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The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(11): 1112-1113

www.thepharmajournal.com
Received: 02-09-2023
Accepted: 11-10-2023

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Variations in RWC, stomatal density and stomata size in pomegranate progenies

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Abstract
Pomegranate (*Punica granatum* L.) has gained recognition as a significant crop with its unique blend of cultural importance and potential health benefits. In this study, twenty pomegranate progenies were assessed, examining key physiological characteristics critical to their growth and development. The parameters evaluated included relative water content, stomatal density, and stomata size. Relative water content, a key factor in maintaining water balance, was examined to gauge the progenies' ability to withstand water stress – a factor with direct implications for crop yield and quality. Stomatal density, another vital aspect of plant physiology, was measured, offering insights into the progenies transpiration rates and water use efficiency. The modulation of stomatal density is influenced by various environmental factors, providing valuable data on their adaptability to changing conditions. RWC values among the progenies ranged from 47.82% to 94.44%. Stomatal density ranges from approximately 47.21 to 223.67 stomata (0.009 µm²) and the stomata size varies from 146.53 to 357.00 µm² among the pomegranate progenies.

Keywords: RWC, stomatal density, stomata size, pomegranate

Introduction
Pomegranate (*Punica granatum* L.) is a perennial fruit-bearing shrub or small tree that has been cultivated and valued for its fruits for millennia. The pomegranate is not only renowned for its exquisite taste but also for its numerous health benefits, making it a significant crop in various regions around the world. Historically, it has been featured in art, literature, and cultural traditions, symbolizing fertility, prosperity, and abundance. Understanding relative water content and stomatal density is crucial in plant physiology, as these factors play a pivotal role in the growth, development, and overall well-being of plants. RWC provides critical insights into a plant's water balance and its ability to withstand drought stress, a factor that significantly affects crop yield and quality. Monitoring RWC helps researchers and growers assess plant water status and guide irrigation strategies, ensuring that plants receive an adequate water supply for optimal growth. Stomatal density is a key determinant of a plant's transpiration rate, which affects its water use efficiency. The density of stomata is influenced by various environmental factors including light, humidity and CO_2 concentration making it a valuable tool in understanding a plant's response to changing environmental conditions. The relationship between relative water content and stomatal density is intricate. Changes in water availability can influence stomatal density and consequently affect a plant's ability to regulate its water balance. Understanding this relationship is vital, as it helps us comprehend how plants respond to water stress, adapt to changing environmental conditions and ultimately influence crop productivity and ecosystem dynamics.

Materials and Methods
Relative water content (RWC)
The relative water contents (RWC) of leaves were measured according to Smart and Bingham (1974) [4]. After sampling, leaf discs were taken and their fresh weight were immediately weighed and then immersed in distilled water for 7 h at ambient temperature. The leaves were then blotted dry and weighed prior to oven drying at 70 °C for 48 h. The leaf relative content was calculated using the formula,

$$\text{RWC (\%)} = \frac{\text{Fresh weight (g)} - \text{dry weight (g)}}{\text{Turgid weight (g)} - \text{dry weight (g)}} \times 100$$

Stomata density and size

Mean stomata density (SD) and stomata size (Ss) were recorded by using fevicol method. Fevicol imprints of the lower leaf surface were observed under Olympus microscope at 40x magnification and expressed in terms of number of stomata per 0.009 sq. µm area of leaf surface (Divya *et al*., 2014) [1]. Stomatal size (length and width) was measured with the same microscope under 40x expressed in µm.

Results and Discussion

The RWC values among the progenies range from 47.82% to 94.44%. Progeny 5/15 has the highest RWC at 94.44%, indicating superior water retention capability, likely contributing to its plant health and growth. Progeny 4/15 has the lowest RWC at 47.82%, suggesting poor water retention, which might impact its overall performance. RWC, may be desirable for arid or drought-prone regions, as they can maintain better water status under water-stressed conditions. Progenies with lower RWC may not be suitable for areas with limited water availability, as they have reduced water-retaining capacity. Breeders and farmers can use this information to select progenies that best suit the water availability conditions in their region, aiming for optimal plant growth.

Stomatal density ranges from approximately 47.21 to 223.67 stomata (0.009µm²). Progeny 3/15 has the highest stomatal density, while progeny 11/7 has the lowest. Stomatal density can be associated with gas exchange, transpiration rates, and response to environmental conditions. High stomatal density can suggest efficient CO_2 uptake. Stomata size varies from 146.53 to 357.00 µm² among the progenies. Progeny 11/7 exhibits the smallest stomata, while progeny 3/15 has the largest. Stomatal size can impact the rate of transpiration and gas exchange. Smaller stomata may reduce water loss but could limit CO_2 uptake. Increase in stomatal density and decrease in stomatal size indicates the adaptation to drought condition. It is also reported that the smaller stomata are better at improving water use efficiency due to their more rapid response to changes in environmental conditions such as humidity (Drake *et al*., 2013) [2]. Similar findings were reported by Dow *et al*. (2014) [3] in olive Smart R, *et al*. (1974) [4] Larbi A. *et al*. (2014) [5].

Table 1: Relative water content and stomatal variation in pomegranate progenies

S.N0	Progenies	RWC (%)	Stomatal density (0.009 µm²)	Stomata size (µm²)
1	2/9	71.42	145.63	260.32
2	2/15	73.68	223.67	207.51
3	3/15	63.63	197.46	357.00
4	3/8	70.00	148.11	262.66
5	4/2	73.33	140.21	245.21
6	4/7	72.41	71.31	167.45
7	4/15	47.82	201.72	298.66
8	5/15	94.44	199.98	300.33
9	9/11	66.66	153.42	336.57
10	10/13	83.41	149.91	243.66
11	11/7	72.35	47.21	146.53
12	11/14	80.76	68.51	200.01
13	11/15	70.37	221.42	158.92
14	12/7	71.87	50.13	157.71
15	12/8	72.41	72.32	197.02
16	12/18	73.33	68.21	202.31
17	14/10	62.96	127.86	298.72
18	15/10	66.66	140.01	246.33
19	15/6	62.51	51.47	223.00
20	18/15	69.31	139.68	253.21

Conclusion

The diverse range of Relative Water Content (RWC), stomatal density, and stomata size observed among the different pomegranate progenies highlights the inherent adaptability and response of these plants to varying environmental conditions These findings underscore the importance of considering RWC, stomatal density, and stomatal size when selecting progenies for specific regions and environmental conditions.

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